

Assessment of various diagnostic methods of ureaplasma respiratory tract infections in newborns*

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We compared three methods used microbial culturing for detection of ureaplasmas in endotracheal aspirate from 500 prematurely born neonates with respiratory disturbances: BioMerieux test, PCR and microbial culturing. Ureaplasmas were detected in respiratory tracts of 79 (16%) newborns. Correlation of the results of culture with those obtained with the BioMerieux kit, culture with PCR and BioMerieux kit with PCR was 97%, 89% and 90%, respectively. Sensitivity and specificity of PCR in comparison with culture was 86% and 98%, respectively, and of the BioMerieux kit 96% and 98%. PCR can be recommended in rapid diagnostics of respiratory infections in newborns suffering from respiratory disorders. It allows the detection of ureaplasmas in case of parallel infections and identification of their species.

Keywords: newborn, ureaplasma, culture, PCR, BioMerieux kit

Ureaplasmas are isolated from the lower genital tract of 60–80% of pregnant women. They are transmitted from mothers to neonates, either *in utero* or during passage through the infected birth canal. Vertical transmission is most frequent in infants of birth weight less than 1000 g. A consequence of ureaplasma infection could be respiratory insufficiency in newborns and other diseases (Gravett *et al.*, 1986; Waites *et al.*, 1988; Lauterbach *et al.*, 1995; Regan & Greenberg, 2001). Respiratory tract colonisation of premature infants has been consistently associated with pneumonia, bronchopulmonary dysplasia and chronic lung disease (Waites *et al.*, 1989; Pacifico *et al.*, 1997; Da Silva *et al.*, 1997).

Culturing of ureaplasmas is difficult and time-consuming. Special culture media and growth conditions are necessary. Routine bacterial cultures are not sufficient to recover *Ureaplasma* species. Isolation by culture is considered the gold standard for detection of ureaplasmas. Unfortunately, it is expensive, requiring special handling and rich media. Furthermore, it may take 2 to 5 days to obtain a result. In

newborns, an additional problem is the necessity of antibiotic administration before the diagnostic procedures are started. Antibiotics inhibit the growth of ureaplasmas, thus limiting the reliability of the culture method.

A search for new methods is ongoing. Such methods should be of a comparable sensitivity and specificity as the classic culture method but at the same time, they should have the advantage of being easy and fast. Therefore molecular techniques are constantly becoming more important in diagnostics. Rapid detection is needed to diagnose infection in newborns. Currently, the polymerase chain reaction (PCR) appears to be the most promising method for detecting these organisms. Recently PCR based methods have been used successfully to distinguish two *Ureaplasma* species. Sequences of the 16S rRNA gene and the 16S rRNA–23S rRNA intergenic spacer regions, genus for urease subunits and the 5' ends of the multiple-banded antigen (MBA) genes are used as targets in PCR-based assays (Kong *et al.*, 2000). At present there are no commercially available molecu-

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Abbreviations: BM, BioMerieux kit (Mycoplasma IST 2); C, culture; PCR, polymerase chain reaction.

lar tests, which could be used in the diagnostics of ureaplasma infections.

The purpose of our study was to compare three diagnostic methods of ureaplasma identification in newborns with respiratory disorders: culture in PPLO medium, a commercial test and PCR with primers, which should differentiate between two species of ureaplasmas.

MATERIALS AND METHODS

Patients. Materials for this study were obtained from 500 newborns with respiratory disturbances hospitalised in a clinic supervised by the Chair of Neonatology, Jagiellonian University Medical College in Cracow (Poland).

Detection of ureaplasmas. Endotracheal aspirates were collected from each infant and placed in BioMerieux transport media. Next, they were subcultured in liquid and solid PPLO media according to the procedure described by Shepard (1986) and parallelly in Mycoplasma IST 2 kit BioMerieux medium (R2). Liquid media and BM test strips were incubated for 72 h at 37°C, solid media for 5–7 days. The growth of microorganisms on liquid PPLO media was observed as a change of colour of the medium (hydrolysis of urea with the release of ammonia, signalled by a colour change of a pH indicator), while on solid media it was the presence of characteristic brownish colonies of ureaplasmas (magnification 125×). Similarly to liquid PPLO, Mycoplasma IST 2 test is based on a colour change reaction, owing to alkalization of the medium in the presence of ureaplasmas. A positive IST 2 test result was observed when the colour of microwells of the diagnostic strip changed from yellow to red.

Isolation of DNA of ureaplasmas. Crude ureaplasma DNA was isolated from R2 medium after 18–24 h of incubation by means of centrifugation (14000 × g, 20 min, 4°C) and denaturation of the sediment in 100 µl of sterile, distilled water at 95°C.

PCR with species-specific primers. PCR was applied to test for genome specific regions of two ureaplasma species (biovars) occurring in man: *U. parvum* and *U. urealyticum*. Primers for *U. parvum* were UPS2c, UPA2c (420 bp), for *U. urealyticum* there were UUS2c, UUA2c (420 bp) (Kong *et al.*, 2000). Forty thermal cycles were performed, each consisting of: 95°C 5 min, 92°C 1.5 min, 55°C 2 min, 72°C 1.5 min, 72°C 8 min. The PCR solution (25 µl) contained: 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTP, 10 pmol primers, 0.5 U *Taq* polymerase, 5 µl target sample. DNA from reference strains was used as a positive control for identification of biovars of ureaplasmas: serotype IV ATCC 27816 (*U. urealyticum*) and serotype

III ATCC 27815 (*U. parvum*). Negative control was distilled water. Amplified products were visualized under ultraviolet light after electrophoresis through a 2% agarose gel containing 3 µl ethidium bromide.

RESULTS

Ureaplasmas were detected in 79 (16%) infants. Positive results of culture, the Mycoplasma IST 2 test and PCR were obtained for 68, 77 and 75 materials, respectively. In 40 cases, culturing was impossible because of accompanying bacterial flora. In these cases, PCR was relevant. In four samples positive results of BioMerieux kit were not confirmed by PCR. Positive result of one sample assessed by PCR did not correspond with the result of culture. Correlation, sensitivity and specificity of PCR and BioMerieux kit compared with cultivation on PPLO media are shown in Table 1.

Table 1. Correlation, sensitivity and specificity of PCR and BioMerieux kit compared with cultivation on PPLO media as the gold standard.

	PCR/C	PCR /BM	BM/C
Correlation	97%	98%	97%
Specificity	98%	99%	97%
Sensitivity	92%	91%	97%

C, culture; BM, BioMerieux kit (Mycoplasma IST 2)

DISCUSSION

In this study, we evaluated and compared the usefulness of various diagnostic methods of ureaplasma infections: PCR amplification, a commercial BioMerieux test and culturing on solid and in liquid media. We applied species-specific primer sets: UPS2, UPA2 (*U. parvum*) and UUS2c, UUA2c (*U. urealyticum*) targeting the ureA-ureB and ureB-ureC gene spacer regions, respectively. UPS2-UPA2 primers were specific for all amplified serovars of *U. parvum*, UUS2-UUA2 were specific for all serovars of *U. urealyticum* (Kong *et al.*, 2000).

It was recently reported that the sensitivity and specificity of cultivation techniques is comparable to the sensitivity and specificity of molecular methods commercial tests currently applied (Blanchard *et al.*, 1993; Abele-Horn *et al.*, 1996; Clegg *et al.*, 1997; Fernandez *et al.*, 1998). Although cultivation is regarded as the gold standard in identifying mycoplasma and ureaplasma infections, it only enables the detection of living cells which multiply in artificial media. When live microorganisms are not present in the specimen or, owing to various reasons, they are not able to survive outside the host, the results obtained by this method may be falsely

negative. This possibility was for example addressed by Teng *et al.* (1994).

It may also be impossible to detect ureaplasmas by culturing method when some other rapidly growing bacteria are present in the specimen. In our study, in as many as 40 (8%) such cases the PCR method was decisive.

The specificity and sensitivity of ureaplasma detection by culturing in PPLO broth were only slightly worse (both 97%) than those of the commercial test. Its specificity compared with the PCR method was also satisfactory (98%), but the sensitivity was found to be much lower (92%). Our results are consistent with other authors' observations that the cultivation method is less sensitive (Teng *et al.*, 1994; Luki *et al.*, 1998). This relatively low frequency of ureaplasma detection by means of culturing may be attributed in part to the difficulties in growing and isolation of these microorganisms.

The results of PCR amplification are less prone to being influenced by the method of specimen collection and handling. PCR analysis is also much quicker; results can be obtained in 1–2 days, whereas it takes 5–7 days in the case of cultivation and 3–5 days when the commercial test is applied. In four cases the positive result of Mycoplasma IST 2 test was not confirmed by PCR. The rapid test results (the BioMerieux test belongs to this category) may sometimes be falsely positive (Rastawicki *et al.*, 2004). Ureaplasmas produce ureases that degrade urea to ammonia and thus, shift the pH of the medium to more alkaline values resulting in a change of medium colour and such change is taken as indicative of the presence of ureaplasmas. However, one must remember that ureaplasmas are not the only microorganisms capable of hydrolyzing urea with urease. In the presence of other bacteria (e.g. *Proteus*, *Klebsiella*) we may also observe alkalization of the culture medium and the result may then be misinterpreted as positive. In four cases in our study, the positive result of the commercial test was not confirmed by the molecular method owing to the fact that the colour reaction was unspecific. The possibility of such misinterpretation can be limited by the use of commercial test strips along with solid media and tracking the growth of ureaplasmas and other flora forming bacterial colonies in the examined specimen. The necessity of applying both tests

increases cost and is time-consuming. The molecular method also is advantageous because it enables rapid species identification, which in the near future may broaden our knowledge about the pathogenicity of both ureaplasma species. It also allows the detection of ureaplasmas in case of accompanying infections, enables identification their species and is the quickest of the three diagnostic method used in our study.

CONCLUSION

PCR, as a highly sensitive and specific methods, can be recommended in rapid diagnostics for respiratory infections in newborns suffering from respiratory disorders.

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